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Contrasting Behavior of Pentafluorophenoxyacetone and Pentafluorobenzyloxyacetone in Electron Impact and Electron Capture Mass Spectrometry

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Towards the goal of finding new ketone electrophores suitable as molecular labels for electrophoric release tags, pentafluorophenoxyacetone (1) and pentafluorbenzyloxyacetone (2) were prepared. Both ketones were evaluated by electron capture (EC) and electron impact (EI) modes of mass spectrometry (MS). By EC-MS, 1 nearly gave a single ion (as desired), whereas 2 gave many ions. This behavior was completely reversed in EI-MS. To account for certain ion fragments in the EC mass spectrum of 2, an anion radical McLafferty-type rearrangement and loss of a carbene neutral were postulated. Electron impact of 1 gave an abundant ion at m/z 117 (C^5F^{3+}), which was suggested to be a diyne cation. © **1998 John Wiley & Sons, Ltd.**

KEYWORDS: electron capture mass spectrometry; electrophore; Fourier transform mass spectrometry; McLafferty rearrangement; carbene

INTRODUCTION

We are developing electrophores as covalent labels for signal-based analytical methods such as DNA sequencing[1] and immunoassays.[2] Ultimately the electrophore-labeled reagent in the assay is measured by chemically releasing the electrophore prior to its detection by electron capture mass spectrometry (EC-MS). We are focusing on ketone electrophores since they can be derived by oxidatively or thermally cleaving an appropriate glycol linkage that connects the electrophore to the reagent of interest. The ideal ketone electrophore for this purpose gives an abundant single ion in the EC mass spectrum (aside from an associated isotope peak). Since EC-MS can measure many different electrophores simultaneously, potentially many electrophore reagents can be combined to enhance the speed and specificity of signal-based assays.

Here we report the synthesis and testing of two structurally similar ketone electrophores, each of which is an acetone derivative. Whereas one ketone has the detection property we seek (it gives nearly a single abundant ion by EC-MS), the other gives many fragmentation products in the EC mass spectrum. Under electron impact (EI) conditions, the fragmentation behavior of the two ketone electrophores is reversed.

The analysis of fluorinated organic compounds by mass spectrometry was reviewed many years ago[3] and a brief summary of the subsequent literature appeared more recently.[4]

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RESULTS AND DISCUSSION

In Fig. 1 is shown the synthesis and structures of the two ketone electrophores that we studied by EC-MS and EI-MS: pentafluorophenoxyacetone (**1**) and pentafluorobenzyloxyacetone (**2**). As seen, **1** was obtained by converting acetone to 1-iodoacetone with iodine and then reacting this intermediate product with pentafluorophenol in the presence of potassium carbonate. Similar reaction of 1-iodoacetone with pentafluorobenzyl alcohol failed to give **2**, as did reaction of acetol in refluxing acetone with pentafluorobenzyl bromide. Synthesis of **2** was accomplished by reacting propargyl alcohol with pentafluorobenzyl bromide, followed by an acidic hydration reaction of the intermediate pentafluorobenzyl propargyl ether.

The fragmentation behavior of **1** and **2** in EC-MS and EI-MS described here (Schemes 1–5) was observed originally on a quadrupole EC/EI-MS instrument. The compounds were later analyzed using an EC/EI-Fourier transform (FT) MS system, which provided high-resolution, accurate mass measurements.

Fewer ions could be seen with FTMS than with the quadrupole instrument. For example, neither compound gave a molecular ion in FTMS, under either EC or EI conditions, and only some of the product ions proposed in Scheme 2 could be seen. This is probably because the ions are detected within a few milliseconds in the quadrupole instrument, whereas the time is closer to 1 s in FTMS. This provides more time for ions to

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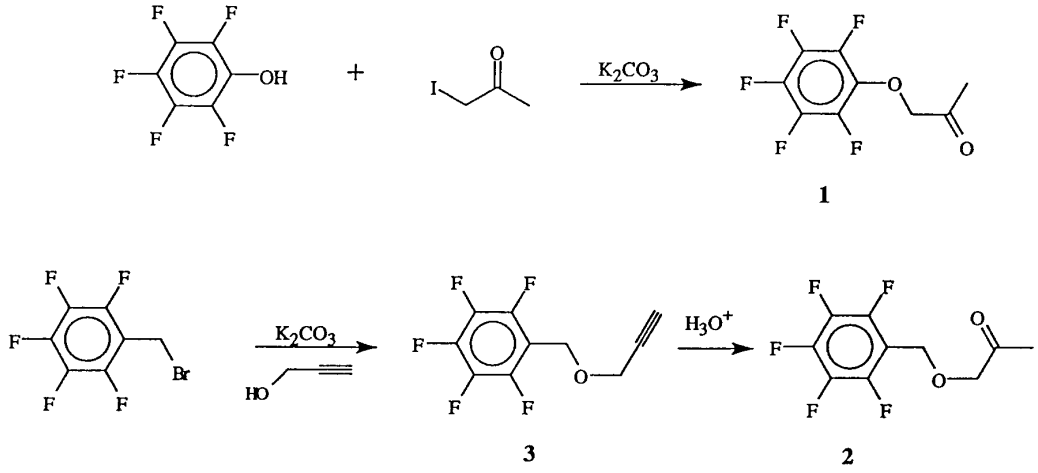


Figure 1. Synthesis of **1** and **2**.

fragment or rearrange in the FTMS instrument. For the ions that could be observed in FTMS, all of the accurate mass measurements were consistent with the elemental compositions that were expected (see Table 1).

Attempts to obtain stepwise fragmentation information by MS/MS experiments with the FTMS instrument were not even partly successful, however. No fragment ions were observed when the parent ion from EC (highest mass ion observed for each compound) was subjected to collisionally induced dissociation (CID). Perhaps this was a consequence, to at least some degree, of electron stripping by CID.[5] In the EI mode, numerous ions were generated from CID on the parent ion, precluding definitive evaluation of the proposed fragmentation pathways.

As seen in Fig. 2(A), **1** gives nearly a single ion by EC-MS, corresponding to a pentafluorophenoxy anion (*m/z* 183) arising from the loss of an acetone radical from the parent anion radical. Additional loss of a fluorine atom explains the minor peak at *m/z* 164.

In contrast, many fragmentation products form when **2** is subjected to EC-MS, as seen in Fig. 2(B). Suggested structures and fragmentation pathways for the major ions are presented in Scheme 1.

A simple loss of a fluorine atom accounts for *m/z* 235, which could, in turn, lose a hydrogen atom, driven by a ring closure, to form *m/z* 234. The latter species also might arise from a single-step elimination of HF. Loss of HF has been reported by others in the analysis of polyfluoroaromatic compounds by EC-MS.[6–8] The fragment ion at *m/z* 215 probably originates from the radical anion $[C_{10}H_6O_2F_4]^{-\cdot}$ at *m/z* 234 by the loss of another fluorine atom. The subsequent transition of *m/z* 234 to *m/z* 214 is the same type of ring closure reaction, including the possibility of two pathways, analogous to the conversion of *m/z* 254 to 234. A simple loss of an acetyl neutral, perhaps involving an electron transfer from the aromatic nucleus to the benzylic carbon of *m/z* 234, explains *m/z* 191. The proposed ring closure products at *m/z* 162 and *m/z* 178 also appear to derive from *m/z* 235. Replacement of an F by OH and O probably explains *m/z* 252 and 251, respectively.

The formation of the fragment with *m/z* 179 is interesting. We suggest that it arises from *m/z* 235 starting with a proton migration followed by loss of an acetyl carbene. This postulated mechanism is shown in more detail in Scheme 2. It is well known that carbanions connected directly to leaving groups, which are named carbenoids, can generate carbenes by an α-elimination process.[9] The released acetylcarbene should finally rearrange to a corresponding methylketene via the Wolff rearrangement.[10] Also shown in Scheme 2 is a fragmentation pathway from the enolate form of *m/z* 235 to account for *m/z* 72 (oxyenolate anion radical) and *m/z* 71 (oxyketene anion derived from *m/z* 72 by loss of a hydrogen atom). This pathway also is summarized in Scheme 1.

We suggest that a McLafferty-type rearrangement is mainly responsible for the formation of the apparent pentafluorobenzaldehyde anion radical at *m/z* 196 (see Scheme 1), as illustrated in more detail in Scheme 3. An anionic McLafferty-type rearrangement has been suggested before.[13] The fragment at *m/z* 196 might also

Table 1. Elemental composition measurement of compounds 1 and 2 by FTMS

Compound	Mode	Formula	Calculated mass (u)	Experimental accuracy (ppm)
1	EC	C_6F_5O	182.9874	3.0
	EI	C_7H_7	91.05422	1.1
		C_5F_3	116.9947	1.3
		C_5F_5	154.9915	1.5
		C_6F_5	166.9915	2.5
		$C_7F_5H_2$	181.0071	1.9
		C_6F_5O	182.9864	3.0
		$C_7F_5H_5O_2$	197.0020	1.6
		$C_9F_5H_5O_2$	240.0112	1.8
2	EC	C_6F_4	147.9941	2.1
		C_6F_5	166.9926	4.2
		$C_7F_4H_2O$	178.0047	2.6
		$C_8F_4H_3O$	191.0125	3.5
		C_7F_5HO	195.9953	1.8
		$C_7F_5O_2$	210.9824	1.5
		$C_{10}F_3H_5O_2$	214.0247	1.2
	EI	C_7H_7	91.05422	1.2
		$C_6F_3H_4O$	149.0209	3.3
		C_7F_4H	161.0009	1.7
		$C_7F_5H_2$	181.0071	1.7
		C_7F_5O	194.9864	1.6

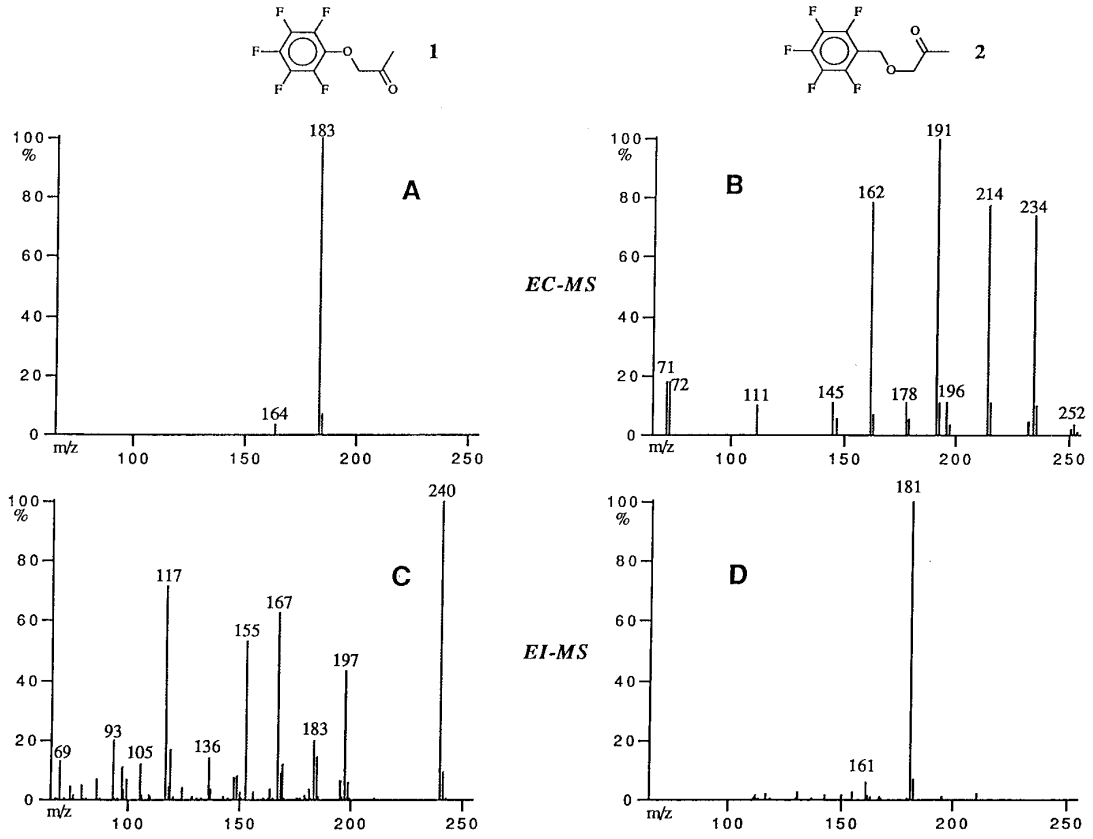


Figure 2. Mass chromatograms of **1** (M_r = 239 Da) by EC-MS and EI-MS (A and C, respectively) and similarly of **2** (M_r = 254 Da) (B and D, respectively).

arise by loss of a hydrogen atom from *m/z* 197, as shown in Scheme 1.

In contrast to its EC mass spectrum, the EI mass spectrum of **2**, shown in Fig. 2(D), is nearly a single peak, at *m/z* 181, which is a pentafluorobenzyl or pentafluorotropylium cation. The weak peak at *m/z* 161 apparently derives from *m/z* 181 by loss of HF. No molecular ion (*m/z* 254) is observed. (However, the positive chemical ionization mass spectrum of **2** displays an $[M + H]^+$ peak at *m/z* 255; data not shown.)

Whereas **1** gives a simple EC mass spectrum, its EI mass spectrum is complex, as seen in Fig. 2(C), which is partly rationalized in Scheme 4. The molecular cation radical gives the most intense peak (*m/z* 240). Single-bond cleavage reactions can explain the fragments at *m/z* 197, 183 and 167. The fragment at *m/z* 167 may also come from the loss of CH_2O from the cation at *m/z* 197 (see Scheme 4), since Russell *et al.*[14] observed a loss of CH_2O from the analogous $C_6H_5OCH_2^+$ ion derived from non-fluorinated phenoxyacetone.

Others have observed three major ions when pentafluorphenol is subjected to EI-MS; *m/z* 184 ($[C_6F_5OH]^{+\cdot}$, the $M^{+\cdot}$), 136 ($[C_5F_4]^{+\cdot}$ and 117 ($[C_5F_3]^+$).[15] Of the minor ions, one appeared to be at *m/z* 155 (our interpretation of the mass spectrum shown in Ref. 15). Interestingly, we also observe major ions at *m/z* 136 and 117, along with an ion fragment at *m/z* 183 ($[C_6F_5O]^+$), plus a major ion at *m/z* 155. We believe that this all can be accounted for by the fragmentation pathways shown in Scheme 5. Apparently the quantitative behavior of the *m/z* 183 and 184 ions initially is different because only the latter can discharge HF. This leads to a major ion at *m/z* 155 from *m/z* 183 that forms only to a minor degree from *m/z* 184, as indicated in Scheme 5. However, once the *m/z* 155 ion losses a fluorine atom, forming *m/z* 136, a common pathway takes place leading to *m/z* 117. Not shown, but conceivable, is that *m/z* 155 might yield *m/z* 117 without proceeding through *m/z* 136. The significant intensity of the *m/z* 117 peak implies a relatively stable species such as the diyne cation shown. Previously a structure for this ion has not been proposed.

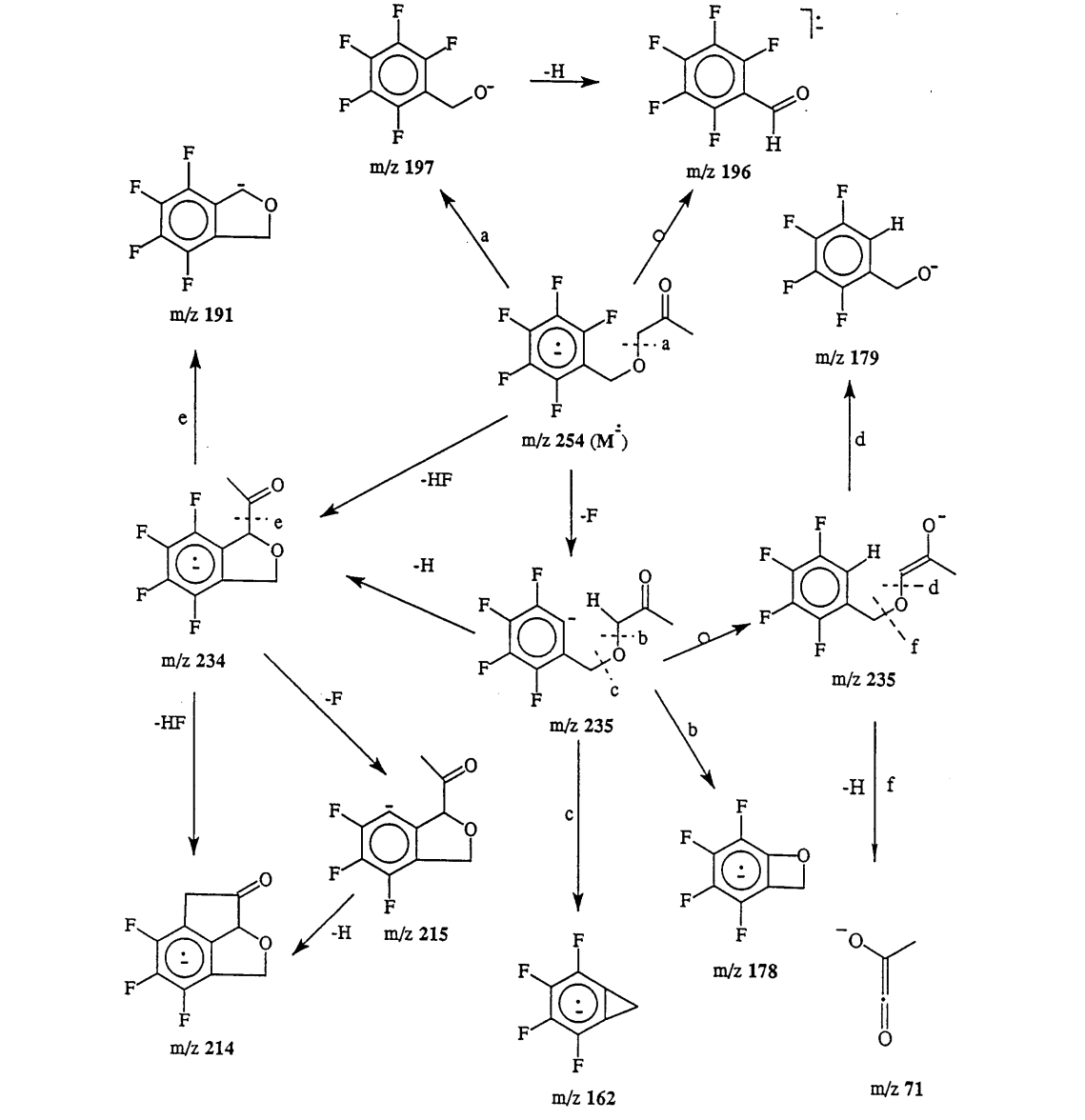
EXPERIMENTAL

Reagents

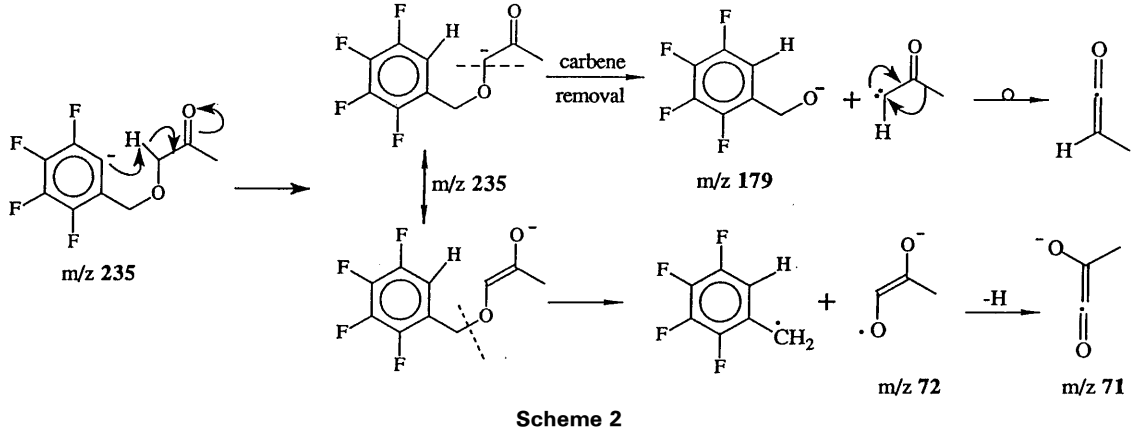
Pentafluorophenol (99+%), pentafluorobenzyl bromide (99+%), propargyl alcohol (99%), triethylbenzylammonium chloride (TEBA), 18-crown-ether and other reagents were purchased from Aldrich Chemical (Milwaukee, WI, USA). All solvents used were obtained from Doe & Ingalls Chemicals (Medford, MA, USA).

Instrumentation

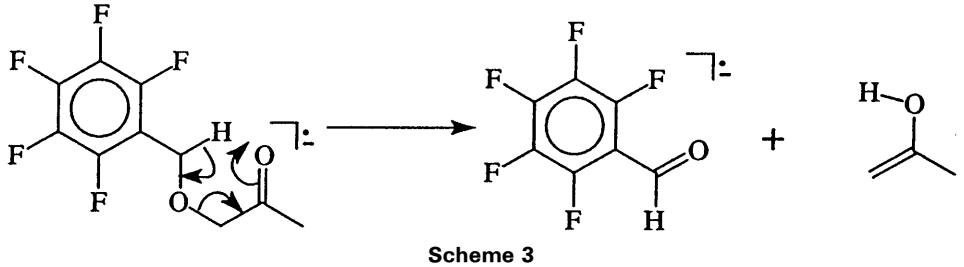
Gas chromatography/electron capture mass spectrometry was performed on a Hewlett-Packard Model 5988A mass spectrometer connected to a Hewlett-Packard Model 5890 Series II gas chromatograph. The



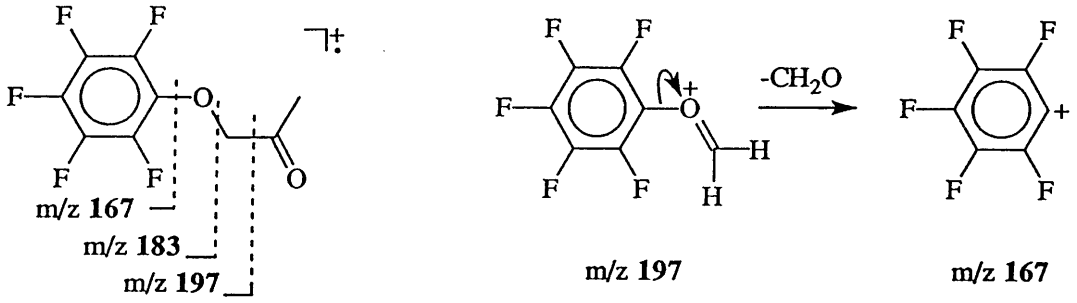
Scheme 1



Scheme 2



Scheme 3



Scheme 4

ion source temperature was 250 °C; the electron energy was 240 eV and the methane ion source pressure was 2 Torr (1 Torr = 133.3 Pa). Helium was the carrier gas for a 25 m × 0.11 mm × 0.17 μm Hewlett-Packard Ultra 1 capillary column. Gas chromatography/electron impact (70 eV) mass spectrometry was performed on a Finnigan Model 4021B quadrupole mass spectrometer interfaced to a Hewlett-Packard Model 5890A gas chromatograph. ^{1}H and ^{13}C NMR spectra were obtained on a Varian XL-300FT instrument with $CDCl_3$ as the solvent and tetramethylsilane as the internal standard. Preparative gas chromatography was carried out on a Varian 3300 instrument with helium as the carrier gas using a 20% SE-30/Chromosorb W (non-acid washed, 60–80 mesh), 10′ × 1/4″ aluminum column.

Elemental compositions of the ions observed in EI-MS and EC-MS were determined by making high-resolution, accurate mass measurements with a Bruker APEX 47e FTMS instrument (Bruker Instruments, Billerica, MA, USA) equipped with an infinity cylindrical ion trap and a 4.7 T superconducting magnet. Samples were introduced through a heated external direct probe insertion port located on the side of the source vacuum chamber. The ion source was kept at 200 °C and the electron energy was 70 eV for both EI and EC. Ions generated at the source were transported to the ICR trap through an electrostatic ion guide. The instrument was calibrated by a two-parameter least-squares fitting procedure using the same experimental conditions. Three ions generated from known ketones synthesized in our laboratory were used as calibration standards at *m/z* 135.0451, 163.0764 and 195.0663, respectively. The mass accuracy was in the low ppm range.

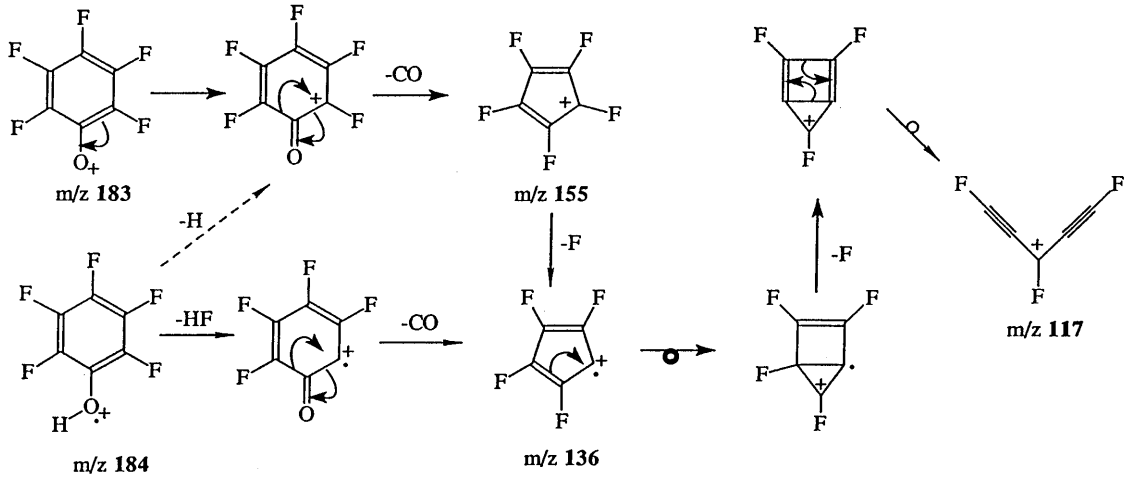
CID was also explored in an unsuccessful attempt to obtain stepwise fragmentation information by MS/MS. Ions of interest were isolated by a sweep pulse which ejected all other ions coexisting inside the ICR ion trap. Nitrogen was introduced into the analyzer by a pulsed valve as collision gas. A resonant excitation pulse (18 μs) was used to achieve CID. No fragment ions were observable for parent ions generated from EC. For the parent ions generated from EI, CID generated numerous fragment ions in the *m/z* region between 80 and 130.

Pentafluorophenoxyacetone (1)

Iodine (2.54 g (10 mmol)) was added to 15 ml of acetone followed by stirring for 2 h at room temperature. The reaction mixture was added dropwise into a stirred mixture of 0.92 g (5 mmol) of pentafluorophenol, 5 g of potassium carbonate, 0.1 g of triethylbenzylammonium chloride and 30 ml of acetone. After refluxing for 3 h, the inorganic substances were removed by filtration. The solvent was evaporated, the residue was dissolved in 30 ml of pentane and 3 g of silica gel were added for decolorization. Preparative gas chromatography gave 0.28 g (23%) of the product as a colorless liquid. ^{1}H NMR, δ (ppm) 2.29 (s, 3H, CH_3), 4.74 (s, 2H, CH_2); ^{13}C NMR, δ (ppm) 26.1 (CH_3), 77.6 (CH_2, $^{4}J_{C,F}$ = 3 Hz), 137.9 (C-2′ and C-6′, $J_{C,F}$ = 250 Hz), 139.0 (C-4′, $J_{C,F}$ = 248 Hz), 141.1 (C-3′ and C-5′, $J_{C,F}$ = 245 Hz), 169.5 (C-1′), 202.8 (C = 0).

Pentafluorophenyl propargyl ether (3)

A mixture of 1.3 g (5 mmol) of pentafluorobenzyl bromide, 0.31 g (5.5 mmol) of propargyl alcohol, 50 mg



Scheme 5

of 18-crown and 5 g of potassium carbonate in 20 ml of acetone was stirred under reflux for 10 h. After the removal of the inorganic substances by filtration, the filtrate was evaporated to remove most of the solvent. Preparative gas chromatography afforded 0.9 g (76%) of product as a colorless liquid. ^{1}H NMR, δ (ppm) 2.51 (t, 1H, ^4J = 2.4 Hz, H—C≡), 4.22 (d, 2H, $^4J_{H,F}$ = 2.4 Hz, CH_2), 4.70 (t, 2H, $^4J_{H,F}$ = 1.8 Hz, CH_2Ar); ^{13}C NMR, δ (ppm) 57.9 (CH_2), 58.3 (CH_2Ar), 75.2 (H—C≡, $J_{C,H}$ = 251 Hz), 78.5 (—C≡), 110.8 (C-1′, $^2J_{C,F}$ = 19 Hz), 137.5 (C-2′ and C-6′, $J_{C,F}$ = 253 Hz), 141.5 (C-4′, $J_{C,F}$ = 255 Hz), 145.8 (C-3′ and C-5′, $J_{C,F}$ = 250 Hz).

Pentafluorobenzyloxyacetone (2)

To a stirred mixture of 0.1 g of sulfuric acid and 1 g of mercury(II) sulfate in 50 ml of methanol–water (3:1), 0.83 g (3.5 mmol) of **3** was added dropwise slowly at room temperature followed by stirring under reflux for 2 h. After the evaporation of most of the solvent, 20 ml of diethyl ether were added. The water layer was separated and the organic layer was dried over anhydrous magnesium sulfate, giving, after solvent removal, 0.83 g (93%) of product as white crystals, m.p. 54–55 °C. ^{1}H NMR, δ (ppm) 2.18 (s, 3H, CH_3), 4.18 (s, 2H, CH_2), 4.74 (t, 2H, $^4J_{H,F}$ = 1.8 Hz, CH_2Ar); ^{13}C NMR, δ (ppm) 25.4 (CH_3), 59.5 (CH_2Ar), 75.2 (CH_2), 110.4 (C-1′, $^2J_{C,F}$ = 18 Hz), 137.2 (C-2′ and C-6′, $J_{C,F}$ = 253 Hz), 141.2 (C-4′, $J_{C,F}$ = 255 Hz), 145.4 (C-3′ and C-5′, $J_{C,F}$ = 253 Hz), 205.1 (C = 0); MS (PCI), m/z (%) 255($[M + H]^+$, 0.5), 181 (100), 161 (9), 58 (25), 44 (22), 43 (58).

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